

REFERENCE LISTING

<131> Binding site for
<132> Carcinoembryonic Antigen (CEA)

<133> Binding site for Carcinoembryonic Antigen (CEA)

<134> Sequence listing: LYX-016.6 US

<140> US 3,941,541

<141> 2000-04-03

<160> 137

<210> 1

<211> 16

<212> PRT

<213> Artificial Sequence

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<221> Description of Artificial Sequence: CEA binding polypeptide

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<223> Xaa is Asn, Asp or is absent

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<222> (5)

<223> Xaa is Asn, Glu or Met

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<222> (6)

<223> Xaa is Asn, Leu, Met or Phe

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<222> (7)

<223> Xaa is Asp, Gly, Ile, Lys, Phe or Thr

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<223> Xaa is Ala, Gln, Gly, Lys or Thr

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<223> Xaa is Ala, Asn, Arg, Leu or Gly

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<222> (13)

<223> Xaa is Arg, Leu, Pro or Ser

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<222> (16)

<223> Xaa is Leu, Ser, Trp or Tyr

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Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa

1

5

10

15

<110> 2

<111> 16

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<222> (1)

<223> Xaa is Asn or Asp

<220>

<221> VARIANT

<210> 1
 <211> Xaa is Ile, Met, Leu or Asn
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 <214> VARIANT
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 <216> Xaa is Arg, Asn, Asp, Glu, Gly or Trp
 <217> VARIANT
 <218> 12
 <219> Xaa is Ala, Gly, His, Phe, Thr, Tyr or Val
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 <222> Xaa is Arg, Leu, Pro or Ser
 <223> VARIANT
 <224> 16
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 1 5 10 15
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 <220> Description of Artificial Sequence: CEA binding
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 <226> Xaa is Asn, Leu, Met or Phe
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<222> (8)

<223> Xaa is Ala, Val, His, Lys or Thr

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<223> Xaa is Ala, Trp or Tyr

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<222> (9)

<223> Xaa is Ala, Gly, His, Phe, Thr or Val

<400> 3

Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys

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5

10

<210> 4

<211> 16

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<220>

<223> Description of Artificial Sequence: CEA binding
polypeptide

<400> 4

Asn Trp Val Cys Asn Leu Phe Lys Asn Gln Trp Phe Cys Asn Ser Tyr

1

5

10

15

<210> 3

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: CEA binding
polypeptide

<400> 1

Asp Trp Val Lys Gln Asn Lys Lys Asp Gln Trp Thr Cys Asn Leu Leu

1

5

10

15

<210> 6
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 polypeptide

<400> 6
 Asn Trp Asp Cys Met Phe Gly Ala Glu Gly Trp Ala Cys Ser Pro Trp
 1 5 10 15

<210> 7
 <211> 16
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: CEA binding
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<400> 7
 Asp Trp Val Cys Glu Lys Thr Thr Gly Gly Tyr Val Cys Gln Pro Leu
 1 5 10 15

<210> 8
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<220>
 <223> Description of Artificial Sequence: CEA binding
 polypeptide

<400> 8
 Asn Trp Phe Cys Glu Met Ile Gly Arg Gln Trp Gly Cys Val Pro Ser
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<210> 9
 <211> 16
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: CEA binding
 polypeptide

<400> 9
 Asp Trp Val Cys Asn Phe Asp Gln Gly Leu Ala His Cys Phe Pro Ser
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<210> 1
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<220> Description of Artificial Sequence: parental
 domain for design of microprotein display library

<221> VARIANT
 <222> (1)..(12)
 <223> amino acid positions 4 and 9 are invariant Cys;
 all other positions Xaa are varied but not Cys, to
 provide a library of 2x10(8) different peptides
 based on the template sequence

<400> 10
 Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
 1 10

<210> 11
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<220> Description of Artificial Sequence: parental
 domain for design of microprotein display library

<221> VARIANT
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 <223> amino acid positions 3 and 9 are invariant Cys;
 all other positions Xaa are varied but not Cys, to
 provide a library of 1x10(9) different peptides
 based on the template sequence

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<210> 12
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<220> Description of Artificial Sequence: parental
 domain for design of microprotein display library

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 <223> amino acid positions 3 and 10 are invariant Cys;

all other positions Xaa are varied but not Cys, to
provide a library of 2.5×10^8 different peptides
based on the template sequence

<210> 13

Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa
1 5 10

<211> 13

<212> 16

<213> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: parental
domain for design of microprotein display library

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<222> (1)..(16)

<223> amino acid positions 4 and 13 are invariant Cys;
all other positions Xaa are varied but not Cys, to
provide a library of 2.5×10^8 different peptides
based on the template sequence

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Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
1 5 10 15

<210> 14

<211> 16

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<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: variable
sublibrary sequence used in designing focused
secondary library

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<222> (1)..(3)

<223> Xaa is any amino acid except Cys

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<222> (5)..(6)

<223> Xaa is any amino acid except Cys

<400> 14

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<210> Xaa is any amino acid except Cys

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<212> 16

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<222> (12)

<223> Xaa is any amino acid except Cys

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<222> (15)

<223> Xaa is any amino acid except Cys

<400> 18

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<210> 19

<211> 16

<212> PBT

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 sublibrary sequence used in designing focused
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<222> 8) .. (11)

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<222> (1)

<223> Xaa is any amino acid except Cys

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<210> 20

<211> 16

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sublibrary sequence used in designing focused
secondary library

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<223> Xaa is any amino acid except Cys

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Xaa Trp Xaa Cys Asn Leu Phe Lys Asn Gln Trp Phe Cys Xaa Xaa Xaa
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<210> 21

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: isolate of
TN10/9 library found not to bind CEA

<400> 21

Asn Trp Arg Cys Lys Leu Phe Pro Arg Tyr Pro Tyr Cys Ser Ser Trp
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<210>
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 <212> PRT
 <213> Artificial Sequence

<214>
 <215> Description of Artificial Sequence: isolate of
 T1773 library found not to bind CEA

<216>
 Arg Lys Cys Glu Asn Phe Pro Trp Ser Leu His Cys Gly Arg Pro
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 amino acid positions in first family of CEA
 binding peptides

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 <223> X is Asn, Leu, Met or Phe

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 <222> (7)
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 <222> (9)
 <223> X is Arg, Asn, Asp, Glu or Gly

<220>
 <221> VARIANT
 <222> (12)
 <223> X is Ala, Gly, His, Phe, Thr or Val

<220>
 <221> VARIANT
 <222> (15)
 <223> X is Arg, Leu, Pro or Ser

<224>
 Asp Lys Val Cys Glu Xaa Xaa Lys Xaa Gln Trp Xaa Cys Asn Xaa Leu
 1 5 10 15

<225> 24

<210> 25
 <211> 27
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: synthetic CEA
 binding peptide with C-terminal immobilization
 sequence

<400> 25
 Ser Asn Trp Val Cys Asn Leu Phe Lys Asn Gln Trp Phe Cys Asn Ser
 1 5 10 15

Gly Ala Pro Gly Gly Glu Gly Gly Gly Ser Lys
 20 25

<210> 25
 <211> 27
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: synthetic CEA
 binding peptide with C-terminal immobilization
 sequence

<400> 25
 Ser Asp Trp Val Cys Glu Asn Lys Lys Asp Gln Trp Thr Cys Asn Leu
 1 5 10 15

Leu Ala Pro Gly Gly Glu Gly Gly Gly Ser Lys
 20 25

<210> 26
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 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: synthetic CEA
 binding peptide with C-terminal immobilization
 sequence

<400> 26
 Ser Asn Trp Asp Cys Met Phe Gly Ala Glu Gly Trp Ala Cys Ser Pro
 1 5 10 15

Trp Ala Pro Gly Gly Glu Gly Gly Gly Ser Lys
 20 25

<210> 27
 <211> 27
 <212> PRT
 <213> Artificial Sequence

<210> 28
 <211> 16
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: C-terminal
 sequence for immobilizing peptides

<400> 28
 Ala Pro Gly Gly Glu Gly Gly Gly Ser Lys
 1 5 10

<210> 29
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 <223> Description of Artificial Sequence: template
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 focused secondary display library

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 <223> X is any amino acid except Cys

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 <221> VARIANT
 <222> (3)..(6)
 <223> X is any amino acid except Cys

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<210> 30
 <211> 16
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 <213> Artificial Sequence

<210> 31
 <211> 16
 <212> PRT
 <213> Artificial Sequence

<220>
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 <223> X is any amino acid except Cys

<410> 31
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<210> 31
 <211> 16
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 sequence for sublibrary used in construction of
 focused secondary display library

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 <223> X is any amino acid except Cys

<410> 31
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 focused secondary display library

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 <223> X is any amino acid except Cys

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 focused secondary display library

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<223> X is any amino acid except Cys

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<222> (9)

<223> X is any amino acid except Cys

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<222> (12)

<223> X is any amino acid except Cys

<220>

<221> VARIANT

<222> (15)

<223> X is any amino acid except Cys

<450> 33

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 1 5 10 15

<210> 34

<211> 16

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 focused secondary display library

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<220>

<221> VARIANT

<222> (9)

<210> 35
 <211> 16
 <212> PRT
 <213> X is any amino acid except Cys

<210> 35
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 1 5 10 15

<210> 35
 <211> 16
 <212> PRT
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<220>
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 sequence for sublibrary used in construction of
 focused secondary display library

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 <222> (1)
 <223> X is any amino acid except Cys

<220>
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 <222> (3)
 <223> X is any amino acid except Cys

<220>
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 <222> (14)...(16)
 <223> X is any amino acid except Cys

<400> 35
 Xaa Trp Xaa Cys Asn Leu Phe Lys Asn Gln Trp Phe Cys Xaa Xaa Xaa
 1 5 10 15

<210> 36
 <211> 16
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: family of CEA
 binding polypeptides

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 <223> Xaa is Asp, Asn, Ala or Ile

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<221> VARIANT

<222> (6)

<223> Xaa is Thr, Leu, Met, Tyr, Ile, Ile or Asp

<221> VARIANT

<222> (7)

<223> Xaa is Asn, Thr or Asp

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<222> (6)

<223> Xaa is Leu, Phe, Tyr, Trp, Val Met, Ile or Asn

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<221> VARIANT

<222> (7)

<223> Xaa is Phe, Leu, Asp, Glu, Ala, Ile, Lys, Asn, Ser, Val, Trp or Tyr

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<221> VARIANT

<222> (8)

<223> Xaa is Lys, Phe, Asp, Gly, Leu, Asn or Trp

<220>

<221> VARIANT

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<223> Xaa is Asn, Pro, Phe, Gly, Asp, Ala, Ser, Glu, Gln or Trp

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<221> VARIANT

<222> (10)

<223> Xaa is Gln or Lys

<220>

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<222> (12)

<223> Xaa is Phe, Thr, Met, Ser, Ala, Asn, Val, His, Ile, Pro, Trp or Tyr

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<222> (14)

<223> Xaa is Asn, Asp, Glu, Pro, Gln or Ser

<221> VARIANT

<222> (15)

<223> Xaa is Val, Leu, Ile, Pro, Ala, Gln, Ser, Met, His, Thr, Lys or Trp

<220>

<221> VARIANT

<222> (16)

<223> Xaa is Leu, Met, Val, Tyr, Ala, Ile, Trp, His,

<211> 41
 Asp Trp Ile Tyr Asn Leu Phe Lys Asn Gln Trp Phe Cys Gln Val Lys
 1 5 10 15

<212> 41
 <213> 41
 <214> PHE
 <215> Artificial Sequence

<216>
 <217> Description of Artificial Sequence: CEA binding
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<410> 41
 Asp Trp Ile Tyr Asn Leu Phe Lys Asn Gln Trp Phe Cys Gln Val Lys
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<411> 41
 <412> 41
 <413> PHE
 <414> Artificial Sequence

<420>
 <421> Description of Artificial Sequence: CEA binding
 polypeptide

<430> 42
 Asp Trp Val Cys Asn Leu Phe Lys Asn Gln Trp Phe Cys Asp Val Met
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<431> 42
 <432> 42
 <433> IPT
 <434> Artificial Sequence

<435>
 <436> Description of Artificial Sequence: CEA binding
 polypeptide

<440> 43
 Asp Trp Met Cys Asn Leu Phe Lys Asn Gln Trp Phe Cys Asp Gln Ile
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<441> 44
 <442> 44
 <443> IPT
 <444> Artificial Sequence

<445>
 <446> Description of Artificial Sequence: CEA binding
 polypeptide

<210> 44

Asp Trp Asp Tyr Asn Leu Ile Tyr Asn Gln Trp Phe Tyr Ile Ala Ile
 1 5 10 15

<211> 16

<212> 16

<213> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: CEA binding
 polypeptide

<400> 45

Asp Trp Ile Cys Asn Leu Phe Lys Asn Gln Trp Phe Cys Asp Ile Arg
 1 5 10 15

<210> 46

<211> 16

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<220>

<223> Description of Artificial Sequence: CEA binding
 polypeptide

<400> 46

Asp Trp Met Cys Asn Leu Phe Lys Asn Gln Trp Phe Cys Asp Val Val
 1 5 10 15

<210> 47

<211> 16

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<220>

<223> Description of Artificial Sequence: CEA binding
 polypeptide

<400> 47

Asp Trp Ile Cys Asn Leu Phe Lys Asn Gln Trp Phe Cys Asp Ala Ile
 1 5 10 15

<210> 48

<211> 16

<212> 1KT

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<223> Description of Artificial Sequence: CEA binding
 polypeptide

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<210> 49
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<211> 16

<212> PRT

<213> Artificial Sequence

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<223> Description of Artificial Sequence: CEA binding polypeptide

<400> 49

Asp Trp Val Cys Glu Phe Leu Lys Met Gln Trp Ala Cys Asn Val Leu
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<210> 50

<211> 16

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<223> Description of Artificial Sequence: CEA binding polypeptide

<400> 50

Asp Trp Val Cys Asn Leu Phe Lys Asn Gln Trp Phe Cys Asn Val Met
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<210> 51

<211> 16

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<223> Description of Artificial Sequence: CEA binding polypeptide

<400> 51

Ala Trp Pro Cys Asn Leu Phe Lys Asn Gln Trp Phe Cys Pro Pro Gln
 1 5 10 15

<210> 52

<211> 16

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<223> Description of Artificial Sequence: CEA binding polypeptide

<400> 52

Asp Trp Val Cys Asn Leu Pro Lys Asn Gln Trp Phe Cys Asp Val Leu

<210> 53
 <211> 16
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<400> 53
 Asp Trp Val Cys Asn Leu Phe Lys Asn Gln Trp Phe Cys Asp Lys Trp
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<400> 54
 Asp Trp Val Cys Glu Trp Leu Lys Met Gln Trp Ala Cys Asn Met Leu
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 polypeptide

<400> 55
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<400> 56
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 1 5 10 15

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 1 5 10 15

<210> 59
 <211> 16
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<220>
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<210> 60
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<220>
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<210> 61
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<214>
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<210> 62
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<214>
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<214>
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<400> 66
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<400> 67
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<210> 68
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<400> 68
 Asp Trp Val Cys Glu Trp Phe Lys Pro Gln Trp Phe Cys Asn Pro Leu
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<210> 69

<210> 17
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<210> 70
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<210> 71
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<210> 73
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<217> 74
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<218> Description of Artificial Sequence: CEA binding
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<219> 75
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<220> 76
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<221>
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<222> 77
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<223> 78
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<224>
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<225> 79
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 1 5 10 15

<226> 80
 <226> 16
 <226> PRT
 <226> Artificial Sequence

<227>
 <227> Description of Artificial Sequence: CEA binding
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<228> 81
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 1 5 10 15

<229> 82
 <229> 16
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<210>

<210> Description of Artificial Sequence: CEA binding polypeptide

<211> 21

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<212> PRT

<213> Artificial Sequence

<220>

<220> Description of Artificial Sequence: CEA binding polypeptide

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Asp Trp Val Cys Gln Tyr Glu Lys Asp Gln Trp Ser Cys Asn Ile Leu
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<210> 83

<211> 16

<212> PRT

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<400> 83

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<210> 84

<211> 16

<212> PRT

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<400> 84

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<212> PRT

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<224> Description of Artificial Sequence: CEA binding
polypeptide

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<224> Description of Artificial Sequence: CEA binding
polypeptide

<224> 16
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<224> 16
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<224> Description of Artificial Sequence: CEA binding
polypeptide

<224> 16
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1 10 16

<224> 16
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<224> Description of Artificial Sequence: CEA binding
polypeptide

<224> 16
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<224> 16
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<210> Description of Artificial Sequence: CEA binding
polypeptide

<211> 16

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<212> PRT

<213> Artificial Sequence

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<223> Description of Artificial Sequence: CEA binding
polypeptide

<400> 90

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<210> 91

<211> 16

<212> PRT

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<223> Description of Artificial Sequence: CEA binding
polypeptide

<400> 91

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<211> 16

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<223> Description of Artificial Sequence: CEA binding
polypeptide

<400> 92

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<210> 93

<211> 16

<212> PRT

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<217> PRT

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<219> PRT

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<221> PRT

<222> Artificial Sequence

<223>

<223> Description of Artificial Sequence: CEA binding
polypeptide

<400> 94

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<225> PRT

<226> PRT

<227> Artificial Sequence

<228>

<228> Description of Artificial Sequence: CEA binding
polypeptide

<400> 95

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<229> PRT

<230> PRT

<231> PRT

<232> Artificial Sequence

<233>

<233> Description of Artificial Sequence: CEA binding
polypeptide

<400> 96

Asp Trp Val Cys Asn Leu Lys Lys Lys Asp Lys Trp Phe Cys Glu Trp Ala
1 5 10 15

<234> PRT

<235> PRT

<236> PRT

<237> Artificial Sequence

<238>

<238> Description of Artificial Sequence: CEA binding
polypeptide

<210> 97

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 1 5 10 15

<211> 16

<212> PRT

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<220>

<223> Description of Artificial Sequence: CEA binding
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<400> 98

Asp Trp Tyr Cys Asn Leu Phe Lys Asn Gln Trp Phe Cys Asp Leu Val
 1 5 10 15

<210> 99

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: CEA binding
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<400> 99

Asn Trp Val Cys Asn Leu Phe Lys Asn Gln Trp Phe Cys Asp Glu Met
 1 5 10 15

<210> 100

<211> 16

<212> PRT

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<220>

<223> Description of Artificial Sequence: CEA binding
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<400> 100

Asp Trp Val Cys Glu Leu Phe Lys Pro Gln Trp Phe Cys Asn Ile Leu
 1 5 10 15

<210> 101

<211> 16

<212> PRT

<213> Artificial Sequence

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<223> Description of Artificial Sequence: CEA binding
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<210> 101
 Asp Trp Val Cys Glu Trp Leu Lys Met Gln Trp Thr Cys Asn Ala Leu
 1 5 10 15

<211> 16
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<400> 102
 Asp Trp Val Cys Asp Tyr Lys Phe Phe Gln Trp Thr Cys Asn Leu Leu
 1 5 10 15

<210> 103
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 <212> PRT
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<400> 103
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<210> 104
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<400> 104
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<210> 105
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<400> 105

<219> Val Tyr Phe Tyr Ser Ala Thr Met Asp Asn Met Leu
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<220> 125

<221> 126

<222> PPT

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<225> Description of Artificial Sequence: CEA binding
polypeptide

<226> 126

Asp Trp Val Cys Glu Phe Phe Gly Met Gln Trp Thr Cys Asn Leu Leu
1 5 10 15

<227> 127

<228> 128

<229> PPT

<230> Artificial Sequence

<231>

<232> Description of Artificial Sequence: CEA binding
polypeptide

<233> 127

Asp Trp Val Cys Glu Tyr Ala Lys Phe Gln Trp Ile Cys Asn Ile Leu
1 5 10 15